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Experimental model of multidirectional disc hernia in rats

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Summary. *We have carried out an experimental investigation of lesions of the intervertebral disc produced by flexion, lateral bending and rotational forces in an attempt to produce disc herniations. Adult Wistar rats were divided into 4 groups: control and posterior, lateral and rotational herniation. There were 10 rats in each group. The tail between the 5th and 8th vertebral segments was used. A Kirschner wire was inserted into each of 2 adjacent vertebrae and the movement produced had an apex which was anterior or lateral depending on the group involved. Variables such as rupture of the annulus, the cellularity of the nucleus pulposus and the site of the lesion in the disc were studied histologically. The height of the disc, the protrusion, the thickness, and the surfaces of the annulus fibrosus and the nucleus pulposus were measured. In every case we found a nuclear displacement which did not become a protrusion. The surface parameters and the cellularity of the nucleus pulposus are most useful indicators and should be included in any study examining the disc after the injection of substances for treatment.*

Résumé. *Nous avons fait un travail expérimental avec comme objectif l'étude des lésions produites dans le disque inter-vertébral par des forces de flexion, de latéralisation et de rotation en essayant de produire une hernie. Le rat Wistar a été l'animal d'expérimentation. Nous avons fait 4 groupes de 10 animaux chacuns (contrôle, hernie postérieure, hernie latérale, hernie rotatoire). L'étude a été réalisée dans la queue du rat entre les 5ème et 8ème vertèbres. Les lésions ont été réalisées par mobilisation de deux vertèbres consécutives à l'aide d'une broche de Kirschner fichée dans chacune d'elle et permettant de faire des mouvements de rotation ou d'angulation à sommet antérieur ou latéral. Histologiquement, ont été étudiées des variables morphologiques comme la rupture de l'anneau, la cellularité et la situation du nucléus dans l'intérieur du disque et des variables morphométriques comme la hauteur du disque, l'épaisseur et la surface de l'anneau et du nucléus, en utilisant un programme informatique d'analyse d'images. Dans tous les cas ont été observés des déplacements du nucléus sans jamais arriver à l'extrusion, les paramètres de surface et la cellularité du nucléus sont d'une grande utilité et peuvent être utilisés comme base pour étude du disque après l'utilisation de substance pour le traitement de la hernie.*

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Introduction

The objective evaluation of the effect of different forces producing disc lesions is essential in studying the mechanics and pathology.

Several experimental models have been used to produce disc herniation with abnormal loading [1–3, 6, 7, 9, 14, 15]. Bipedal rats have been used to reproduce the human situation [15], the maintenance of forced bending of the spine has been studied [9, 11] and cyclical changes have been

Table 1.

	Control group	Posterior group	Lateral group	Rotational group
Discal height (mm)	1.219	0.895	1.002	0.852
Annular protusion (mm)	0.079	0.253	0.581	0.44
Annular thickness (mm)	0.611	1.123	1.947	1.633
Nuclear surface (mm^2)	0.74	0.993	0.184	0.307
Annular surface (mm^2)	2.63	2.309	3.179	3.085
Back movement (degrees)	35.5	29.4	35.2	28.5
Lateral motion (degrees)	25.4	27	17.4	17.3
Rotational motion (degrees)	14.3	1.3	7.1	4.1

applied to the disc [1, 7, 9]. Others have used direct action on the disc by damaging the annulus [8], increasing disc pressure by the injection of liquid [11], or resection of the posterior arch to produce instability [13]. Takenaka produced an acute disc hernia by bending the rat's tail [14], but this method is difficult to reproduce or quantify.

The aim of our work is to use an experimental model which will reliably reproduce disc herniations, and to evaluate the structural and histological changes [5, 10, 11, 17].

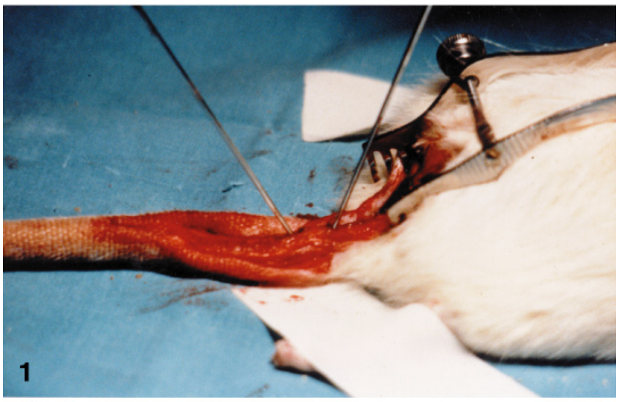
Materials and methods

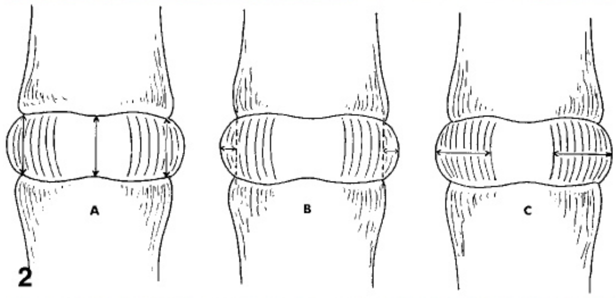
We used the tails of adult Wistar rats of either sex weighing between 350 and 450 kg. The tail has 26 to 28 distinct segments which are easily distinguished and the 5th to the 10th were used because of their homogeneity, mobility and the absence of degenerative changes. The tendons and ligaments at this level are distributed longitudinally on the front, back and both sides of the vertebrae. Their insertion is particularly important posteriorly and their resection weakens the posterior arch.

The rats were divided into 4 groups of 10 each.

- Controls. A circumferential resection of ligaments and tendons was carried out at the level studied.
- Posterior herniation. The spine was flexed to 40° and the posterior tendons and ligaments were divided.
- Lateral herniation. The spine was flexed laterally to 30° with division of the lateral tendons and ligaments at the apex of the curve.
- Rotational hernia. The spine was rotated to 20° with circumferential division of tendons and ligaments.

To carry out these manoeuvres, 2 Kirschner wires, 10 cm long and 1.5 cm thick were inserted into adjacent vertebral bodies making the disc the apex of the angle produced (Fig. 1). The extent of the angle achieved in each group ensured that it was similar, or slightly greater, than that in the controls (Table 1).





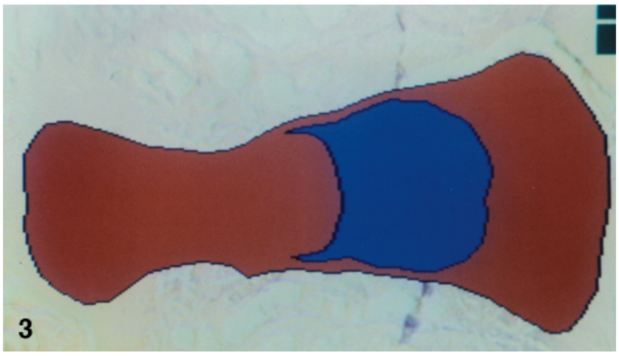


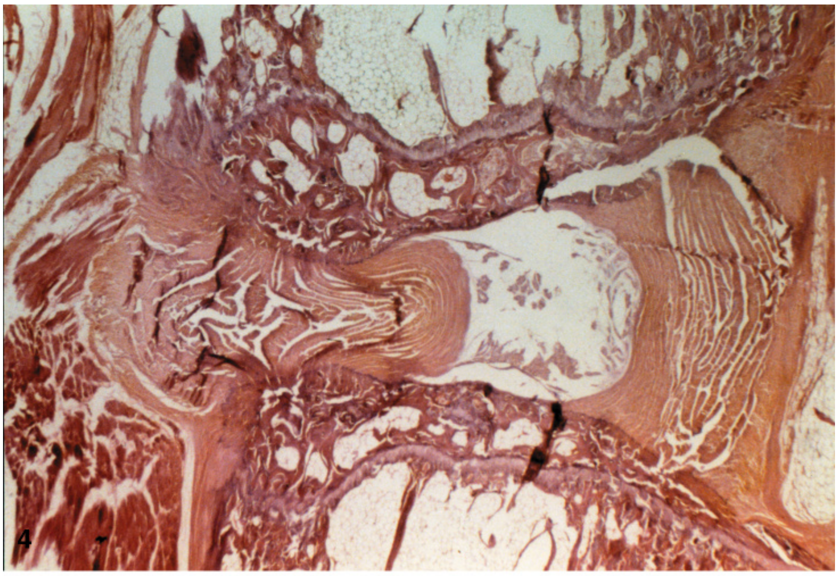
Fig. 1. The method of producing a posterior herniation

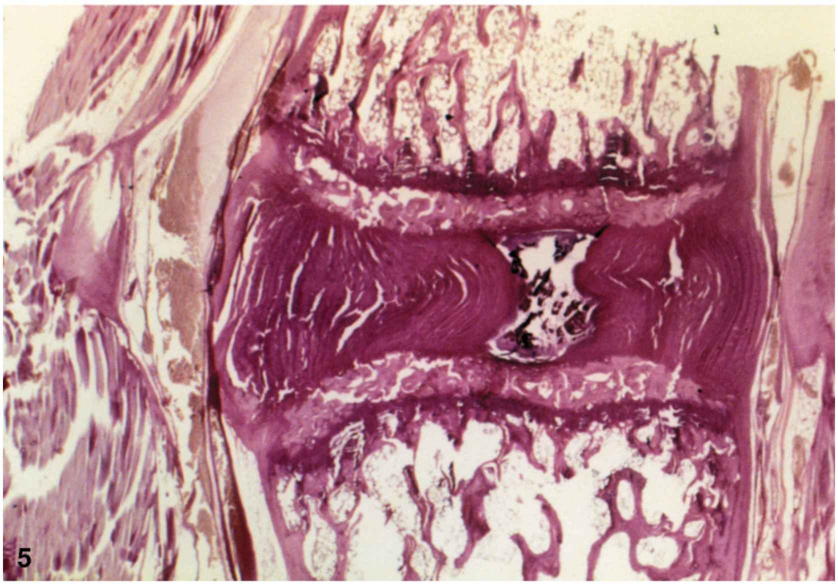
Fig. 2. A–C. Linear measurements of the intervertebral disc. **A** The height measured at 3 points in the disc; **B** protrusion of the annulus, and **C** the thickness of the annulus

Fig. 3. The computerised method used to measure the discal surfaces in a posterior herniation

The rats were killed after 2 weeks and the damaged segment, including both the disc and both vertebral bodies, removed. After decalcification with nitric acid, the specimen was embedded in paraffin, cut in the plane of the disc and stained with haematoxylin-eosin and PAS.

The height of the disc, annular protrusion, the surfaces of the annulus and nucleus pulposus, and the intersegmental mobility were studied (Fig. 2, 3). Stability was tested with the two Kirschner wires in the same way as the disc lesions were produced. Measurements were made on the histological preparations and quantified using Sony MX-10 processing equipment with software designed in our university. Measurement of discal height has been made at the central and peripheral zones (Fig. 2), the final value for each rat being their mean. The protrusion and thickness of the annulus has





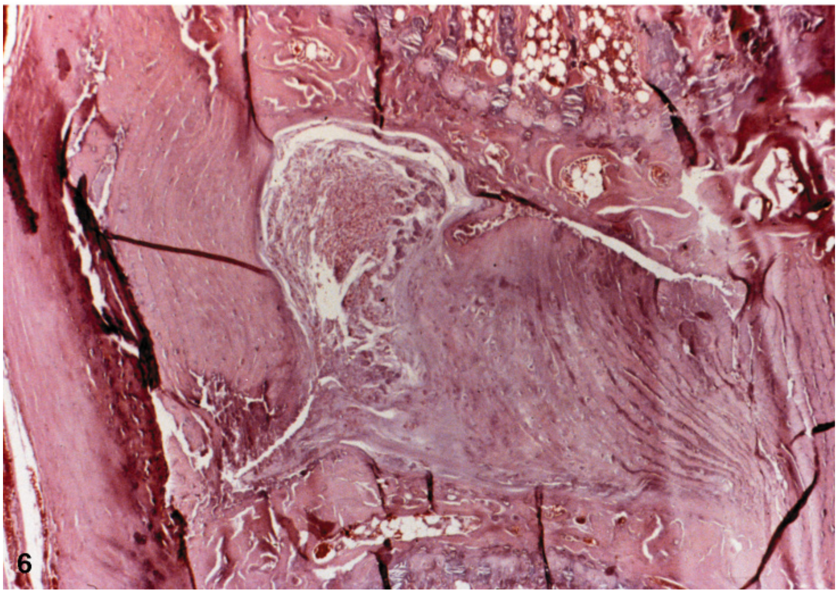


Fig. 4. The histological appearance of a posterior herniation showing an increase in thickness and of the surface of the annulus with a decrease in cellularity of the nucleus pulposus. There is a difference in height on the side of the herniation. HE, ×2

Fig. 5. The histological appearance of a lateral herniation with increased thickness and surface of the annulus with a decrease in the surface of the nucleus pulposus. PAS, ×2

Fig. 6. The histological appearance of a rotational herniation showing the eccentric and decrease in surface of the nucleus pulposus. HE, ×2

been measured in the posterior and lateral herniations; in the rotational on the side next to the nucleus, and in the control group on both sides (Table 1).

In considering the structural changes, we have observed whether there was an annular tear, a vertebral fracture or discal infection, and the site of the protrusion and cellularity of the nucleus pulposus. The site may be central or peripheral if the nucleus is near the bony endplate or the annulus.

Measurement of the number of cells depends on the cellularity of the nucleus pulposus:

· Normal – 500 cells/nucleus pulposus.
· Intermediate – 25–250 cells/nucleus pulposus.
· Haemorrhagic – when there are 3 or more areas of haemorrhage in the nucleus pulposus.
· Zero – when there are less than 25 cells.

The results have been analysed statistically with a Macintosh II computer with a Statview programme, using Mann-Whitney's U-test, contingence tables and a correlation test. Statistical significance was $P = 0.05$ and a correlation test of $c = 0.250$.

Results

In every case there was a change in the position of the nucleus within the disc which was eccentric compared to its original central position. The nucleus was never extruded in spite of breaks in the annulus. These breaks were found in 70% of lateral herniations and 60% of rotational, but never in posterior herniations. Herniation was peripheral in all cases: in posterior herniations it was towards the annulus, in lateral herniations towards the endplate, and in rotational herniation 30% were towards the annulus, 10% towards the endplate and 60% towards the intermediate zone.

Cellularity was never normal; it was intermediate in all posterior herniations and in 50% of rotational herniations; haemorrhage was present in 50% of lateral herniations.

There were 5 discal abscesses and 3 vertebral fractures evenly distributed between the groups.

There was a decrease in discal height in all groups (Table 1), most occurring in rotational herniations ($P = 0.002$). In posterior herniations the measurement of discal height at 3 levels showed asymmetry which was not found in the other groups. The mean value of annular protrusion was four times higher than in the controls, with the highest values in lateral herniations (Fig. 5), although this was not statistically significant.

There was, however, a significant increase in annular thickness which was greatest in lateral herniations. The values for protrusion and annular thickness in posterior herniations are lower because the inner annular fibres are turned inwards towards the nucleus pulposus, producing a cystic area in the thickness of the fibrous annulus (Fig. 4).

Measurements of the surface of the nucleus pulposus showed a decrease in lateral and rotational herniations and an increase in posterior ($P < 0.01$), (Fig. 6). The findings on the annular surface are opposite to those on the nucleus pulposus, being higher in the latter and lower in the former. Segmental mobility is limited on the side where the herniation occurred.

Discussion

The development of intervertebral disc herniation has been reported as the result of a single traumatic episode [1, 6]. Changes in the disc are only seen when forces are greater than the normal limit [2]. Two methods of investigation have been used. Firstly, where the mobile segment is submitted to cyclical multiaxial forces [1, 6, 7], but cadavers have been used so that the reaction of the tissues cannot be studied [6]. The second evaluates the direct effect of mobility on a disc with normal bending [8, 13], but the direct action on the disc could alter the findings. We have only interfered with the posterior arch structures, but the facets are left intact [3] which might be responsible for the differences in experimental herniations [15].

The typical herniation is posterior, which is different from the other 2 groups, and this determines the different behaviour of the annular fibres in the different quadrants. This could be due to variation in structure of the fibres, or associated with altered biomechanics, or different types of insertion of the annular fibres into the peripheral vertebral border [12, 15]. Compression of human discs behaves in a similar way [3].

In rotational herniations there is a greater decrease in discal height associated with the pathological effect on the disc when the protection of the posterior articular facets is removed [4, 12].

Major protrusions occur in discs where the discal height is slightly reduced, as in lateral herniations. This diminution in height is associated with major protrusions where there may be other factors, such as annular fractures which occur in lateral herniations. There are also fewer annular fractures in posterior herniations because of the inversion of the annular fibres and cyst formation in its inner part [3, 9, 14, 16, 17].

We have found limitation of mobility in the herniated segment [13], which could correspond to increased forces applied through the spinal muscles [11]. An association between annular fracture and discal protrusion has already been described [6, 17], but this may not be correct because of possible histological artefacts [5].

There is decreased nuclear cellularity in all the herniations we have produced which corresponds to the relative lack of cells in human discs [5]. There is no agreement about the cellularity in experimental models. The closest model to our own [14] shows a global, but unquantified, decrease in viable cells, with cellular regeneration in the long term [8, 10].

The surface of the nucleus pulposus behaves differently after there has been a herniation; lateral and rotational herniations show a diminution of the surface [2, 3, 8, 14], but in posterior herniations there is an increase compared with the controls.

Our study is limited by the histology in one plane as the herniation may be eccentric and irregular.

Our model is valid in the study of disc herniation; the numerical surface parameters are most useful as they indicate most discal components. The cellularity of the nucleus pulposus shows the viability of the disc after external injury and could be the basis for further studies of the behaviour of the disc after the injection of substances used in treatment.

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